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(54) Composition containing inulin or oligofructose for use in cancer treatment

(57) The present invention concerns a composition comprising a functional ingredient chosen among the group consisting of inulin, oligofructose and/or their derivatives, and its use for carcinogenesis prevention and/or cancer treatment.

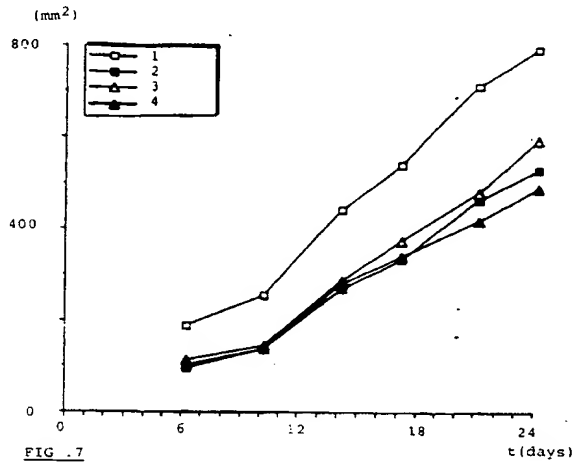

FIG .7

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Description

Field of the invention

The present invention relates to a composition comprising a functional ingredient chosen among the group consisting of inulin, oligofructose and/or their derivatives.

The invention also relates to its use for the manufacture of a medicament destined for the carcinogenesis prevention and/or cancer treatment.

Background of the invention

In industrialised countries the second cause of death after heart disease is cancer. Particularly lung, breast and colon cancer predominate in those countries.

Cancer is the result of an uncontrolled local proliferation of cells with invasion of adjacent normal structures. Metastasis occurs when the cancer spreads via bloodstream or lymphnodes or within a body cavity.

Particularly for breast cancer, most patients are women but male breast cancer occurs with 1% the frequency of female breast cancer. Domestic mammals such as dogs, horses, etc... are equally susceptible to mammary cancer (breast cancer is also known as mammary cancer and interchangeably used in the text herewith). Cancer is a disease which can result from several factors such as ionising radiation, diet, inheritage or exposure to genetic mutagens. Since the determination of the initiation of the breast carcinogenic process is difficult, it is equally difficult to know the exact agents causing the disease.

Although the exclusion of risk factors is a generally accepted approach to avoid the onset or the appearance of putative tumours after the latency period is ended, it is clearly insufficient.

As ideas on the pathogenesis of the disease and on the impact of different factors such as enumerated herein above are continuously evolving, there is still a need for improved compositions and methods to prevent or treat cancer patients.

Moreover, there is an urgent need for malignant tumour prevention due to the high medical costs involved once an individual becomes a cancer patient. Above that, any possible preventive habit which could be used to avoid or at least retard the disease should be investigated.

State of the art

P. D. Cooper et al., Molecul. Immunol. 23(8), (1986), p 895 describes the activation of the alternative pathway of complement by a specific polymorphic form of dahlia inulin named gamma-inulin. Gamma-inulin is formed by >8000 to 10 000 MW polymers (degree of polymerisation 52 to 65) and is insoluble in diluted suspension at 37° C. It is known that an activator of the alternative pathway of complement can have a potential non-specific anti-tumour effect. As such, intraperitoneally injected gamma-inulin is shown to prolong the survival of melanoma bearing mice (P. D. Cooper et al., Molecul. Immunol. 23(8), (1986), p 903)but the timing of the treatment is very critical.

The patent application JP 60/89427 describes an inulin extract from the roots of a specific member of the Campanulaceae, *Platycodon grandiflorum* A.DC. used to treat tumour cell bearing mice. *Platycodon grandiflorum* comprises sapogenins, known for their pharmaceutical properties.

In L. A. Cohen et al., J. Nat. Cancer Inst. 83(7), (1991), p 496 it is described how dietary fibre in a high-fat diet is found to be protective against breast cancer. A supplemental soft white wheat bran exerted an inhibitory effect on the promotional phase of N-methylnitrosourea (MNU) induced breast carcinogenesis in rats when supplemented to a high-fat diet but not when added to a low-fat diet. The researchers speculated that dietary fibre acts by decreasing the digestibility of fat, thus mimicking a low-fat condition.

Summary of the invention

The present applicant has now found that inulin, oligofructose and their derivatives have properties of value as a functional ingredient in the prevention of carcinogenesis, preferably mammary carcinogenesis or treatment of cancer, preferably breast cancer.

The present invention concerns a pharmaceutical composition comprising a functional ingredient chosen among the group consisting of inulin, oligofructose and/or their derivatives.

According to a preferred embodiment of the present invention, said composition is an OTC or a pharmaceutical compound composition eventually comprising conventional vehicles.

According to another preferred embodiment of the present invention, the composition is a functional food or feed possibly comprising additional food or feed ingredients.

Advantageously, said inulin is a chicory inulin, preferably chicory inulin, and more specifically the inulin characterized

by the chromatogram of figure 1 described hereafter.

Preferably, the oligofructose of the composition is obtained by an enzymatic hydrolysis of inulin from chicory, Jerusalem artichoke or dahlia, preferably native inulin, and more specifically the inulin characterized by the chromatogram of figure 1 described hereafter.

In a particular embodiment, the composition comprises also conventional chemotherapeutic products actively destroying malignant tumour cells. Said conventional chemotherapeutic products are described in the "Répertoire Commenté des Médicaments" of the Centre Belge d'Information Pharmacothérapeutique (pages 249 to 253, 1989).

Said chemotherapeutic products are preferably chosen among the group consisting of :

- alkylating compounds which possess alkyl groups highly reactive to specific biomolecules such as DNA (such as chlorambucil, cyclophosphamide, melphalan, carmustine, lomustine, busulfan, cisplastin, thiotépa, chlororiméthin, Ifosfamide, carboplastin, ... and their derivatives),

- antimetabolites which are used instead of the nucleic acids metabolites by the tumour cell (such as methotrexate, cytarabin, fluorouracil, mercaptopurin, thioguanin, azathioprin, hydroxy-carbamide, ... and their derivatives),

- antimitotic antibiotics (such as daunorubicin, bleomycin, mitomycin, ... and their derivatives),

- antitumoral alcaloïds (such as vinblastin, vindesin, vincristin, ... and their derivatives),

- interferons (preferably interferon alpha),

- hormones and antihormones (such as fosfestrol, polyestrodiol, testolactone, tamoxifene, ... and their derivatives) and

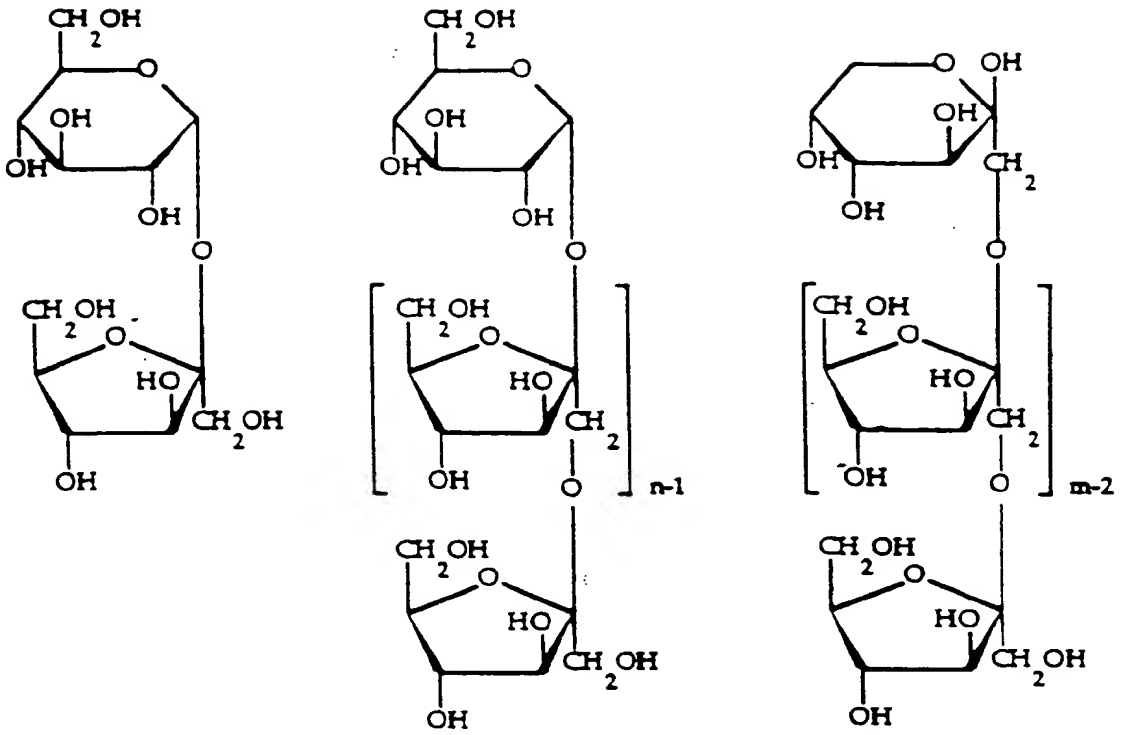
- other specific therapeutic products (such as amsacrin, teniposide, procarbazin, etoposide, ... and their derivatives).

In this context, "functional ingredient" is meant to be a product which should at least modulate carcinogenesis and/or improve the treatment of cancer.

The functional ingredient according to the invention is chosen among the group consisting of inulin, oligofructose or their derivatives.

The inulin used according to the invention is a carbohydrate belonging to the group of polysaccharides named fructans. Fructans are compounds in which fructosyl-fructose linkages constitute the majority of the linkages. In inulin most linkages are of the beta-D-(2->1) fructosyl-fructose type. Most but not necessarily all inulin molecules contain a glucose moiety at the non-reducing end of the chain. Glucose is linked as in sucrose by an alpha-1 -> beta-2 linkage.

It was shown that one of the most suited types of inulin to be used according to the invention, independent of its origin, is basically a polydispersed mixture of very slightly beta-(2->6) branched beta-(2->1)fructan molecules. As most of the molecules have a terminal glucose unit, this inulin can be presented by the general formula GFn (G=glucose, F=fructose, n varies from 2 up to over 60 with a maximum of 72). The present applicant observed that this inulin comprises a small fraction of molecules with general formula Fm that do not contain any glucose at all. The corresponding general chemical formula is thus as follows:



Sucrose **GF_n** **F_m**

GFn are non reducing molecules, all the fructose units are in the furanose form. Fm molecules are reducing. As suggested by A. D. French, J. Plant Physiol. Vol 134, (1989), p 125, the reducing fructose residues are predominantly in the pyranose form when dissolved in D_2O and when the beta-D-(2->6) fructofuranosyl is absent. This was confirmed by NMR analysis by the present applicant to be the case for an inulin particularly suited to be used according to the invention namely native chicory inulin.

In the context of the present invention, "native" shall mean inulin or chicory inulin which prior to its use is extracted from inulin comprising plants with hot water, taking precautions to inhibit plant-own inulinase activity and to avoid acid hydrolysis. The extraction process does not essentially change the molecular structure or the polydispersed composition of native inulin. Native inulin can be extracted from J. artichoke, chicory, dahlia etc.

After starch, inulin is one of the most abundant polysaccharide found in nature. As it is present in cereals (e.g. wheat, barley), vegetables (e.g. onion, leek, garlic, asparagus, salsify) and fruit (e.g. bananas), it is naturally present in our diet.

Degree of polymerisation (DP) is defined as the number of monomer units in one molecule. The average degree of polymerisation (average DP) of inulin depends on the plant source and on the moment of harvesting this plant. As such, native dahlia inulin has an average DP of 20 and native chicory inulin of 11. The percentual distribution of the different molecules can be presented as follows:

	DP*			
	2-9	10-20	21-40	>40
chicory	31	24	28	17
dahlia	13	18	21	40
J.artichoke		52	22	20	6

* Pranzik, J. Chromato. 348,(1985),p 187

Two inulin comprising crops are very well suited for commercial exploitation, namely, Jerusalem artichoke *(Helianthus tuberosus)* and chicory *(Cichorum intybus)*. These plant sources, and the type of inulin they contain, have been studied extensively in the past several decades.

The preferred native inulin of the present invention is chicory inulin. Its average DP varies strikingly in function of the harvesting date. In chicory roots, inulin functions as an osmoregulator increasing cold resistance when broken down. Early in the harvesting season, native chicory inulin has the highest DP. Fig 1 gives a typical HPAEC chromatogram for native chicory inulin suited for the invention and with an average DP of 11,7 and a maximum DP of 72, obtained with a Carbopac PA1 column, a Dionex 4500i with PAD detection and a 100 mM NaOH/100 to 400 mM NaAc elution gradient. The average DP is determined by the fructose to glucose ratio after complete hydrolysis with SP230 Novozym inulinase at pH 4.5, 30 min at 60 °C.

By means of HPLC analysis (see fig 2) it is only possible to differentiate between $DP5^+$ (the integrated sum of DP5 and higher DP molecules), DP4, DP3, saccharose (GF), F2, G, and F.

The DP4 and DP3 fractions are coelutions of GFn and Fm compounds.

CGC, as HPAEC analysis allows to differentiate between GFn and Fm molecules. As shown in fig 3, this method allows to determine oligomers with a DP up to 10 due to a temperature programming starting at 105 °C with a 10 °C increase per min, up to 440 °C. Helium is used as carrier gas at a constant flow rate of 9 ml/min. The native inulin sample is first dried together with phenyl-beta-D-glucopyranoside as internal standard. After treatment with hydroxylamine, the sugars are derived with trimethylsilylimidazole and the volatile derivatives are extracted with isooctane. The determination of the response factor is based upon the analytical results of maltodextrin.

CGC allows to make quantitative analysis of the polydispersed native inulin mixture up to DP 12.

At present, HPAEC is a handsome method to qualitatively characterise inulin but since no standards are available in sufficient quantities, no response factors can be determined and no quantitative measurements could be obtained so far.

The native chicory inulin particularly preferred in the present invention is a 1 to 2 % branched molecule. In the same way, a 4 to 5% branched inulin molecules are present in the particularly preferred native dahlia inulin. The type of linkage and the occurrence of branching is checked by permethylation as described by S. I. Hakamori, J. Biochem. vol 55, (1964)and followed by reductive cleavage and in situ acetylation. The method is based on procedures described by I. Ciucanu et al., Carboh. Res. Vol 131 (2), (1984), p 209; P. Mischnick et al., Carboh. Res. Vol 185(1), (1989), p 113 and J.G. Jun, Carboh. Res. Vol 163(2), (1987), p 247.This technique allows to differentiate 4-linked aldopyranosyl groups from 5-linked aldofuranosyl compounds as the ring structure of each monosaccharide remains intact. By ionic hydrogenation of all glycoside linkages of the methylated polysaccharide one gets partially methylated anhydro alditols that are acetylated in situ.

Fig 4 shows a CGC chromatogram of native chicory inulin treated as such.

A commercially available product comprising native chicory inulin and corresponding to particularly preferred type of inulin suited for the present invention, is Raftiline ® ST or GR (Raffinerie Tirlemontoise, Belgium). The average DP of Raftiline ® ST or GR ranges from 5 to 14, more specifically from 8 to 12, and particularly an average DP between 9 and 11 is preferred. More than 50 % of the molecules in Raftiline ® ST have a DP between 2 and 20 of which more than half have a DP lower than 10. The dry weight of Raftiline ® is on average composed of 92 % of native inulin, 2% of

monosaccharides (G,F) and 6 % of disaccharides (saccharose).

Raftiline ® is poorly soluble : at high concentrations a temperatures above 85 °C is needed to completely dissolved it. At room temperature an 11 % Raftiline ® solution precipitates and the precipitation accelerates and becomes more complete when the solution is cooled down.

On the other hand, Raftiline ® becomes more soluble in hot water but then the pH of the solution becomes an important factor. When the solution is too acidic (pH under 4,5) Raftiline ® is partially hydrolysed into his monomers. When the pH is too high (above 6.5) the solution tends to colour. Fibruline ® standard (Cosucra, Belgium) is another commercially available native chicory inulin comprising product that can be used according to the invention.

A preferred derivative of inulin is a polydispersed inulin essentially free of its mono- and disaccharides. Such a functional ingredient can be obtained according to the process described in PCT/ BE93/00072, included herein by reference. Raftiline ® LS (Raffinerie Tirlemontoise) is a commercially available product comprising such inulin.

Another preferred inulin derivative that can be used according to the invention is the instant inulin which is native inulin dried such that inulin can be added to high dry weight products. The patent application PCT/BE94/00019 describes an instant inulin production method and is included herein by reference. Raftiline ® ST gel comprising instant inulin is commercialised by Raffinerie Tirlemontoise, Belgium.

It can be foreseen to use polydispersed inulin whose average DP is modified compared to the native inulin. This can be obtained either by physically separating off the low DP fractions of the polydispersed mixture, by selectively purifying specific DP fractions, by synthesing longer inulin chains etc. Examples of such inulin comprising compositions are inulin I 2255, I 3754, I 2880 respectively comprising modified average DP inulin prepared from native chicory, dahlia and J. artichoke inulin (Sigma, USA). Another example is polyfructans prepared as described in EP 532 775.

Fructooligosaccharides with DP up to 20 are called, by definition, oligofructose. These saccharides are well soluble in water (up to 80% DW solutions are stable). Oligofructose can be composed of a few percentages of Fm to roughly equal accounts of Fm or GFn molecules.

Onion comprises mainly native oligofructose from which it can be extracted. Oligofructose can be produced from inulin by partial hydrolysis, enzymatic or acidic.

An oligofructose particularly of interest when used according to the invention can be obtained by partial enzymatic hydrolysis of inulin from chicory, J. artichoke or dahlia, preferably native inulin. The DP of the molecules in a preferred polydispersed polydispersed mainly beta-(2->1) oligofructose varies between 2 and 8. The average DP ranges from 3 to 6, preferably 3,5 to 5.

The enzymatic hydrolysis can be executed with an endo-inulinase obtained from Aspergillus, Penicillum or Bacillus. An example is described by B. E. Norman, Denpun, Kagaku 36 (2),(1989), p 103.

A chicory inulin containing extract is partially purified by means of carbonation and filtration. Filtered clarified juice is evaporated up to a concentration suited for the enzymatic treatment, prior to entering the final purification step. The transformation of inulin into oligofructose is achieved by means of an endo-inulinase which is added to the juice and which performs its hydrolytic action. The resulting product is evaporated in order to prevent microbial development. This crude oligofructose is the feed stock for the final purification process carried out by means of demineralisation ion exchange columns.

A final purification of the oligofructose is achieved by an activated carbon treatment which allows to physically adsorb some minor compounds which could not be eliminated in the preceding purification steps. After removing the activated carbon by means of filtration and a final purification on a mixed bed resin, the thus obtained very pure syrup is filter sterilized, prior to evaporation to a commercially defined concentration.

A particularly preferred oligofructose comprising product useful in the present invention is Raftilose ® (Raffinerie Tirlemontoise, Belgium). It is a commercially available product comprising oligofructose obtained by hydrolysing partially purified native chicory inulin using an endo-inulinase.

Another method of preparing the functional ingredients according to the present invention is the reaction of fructosyl transferase with sucrose to bound fructose monomers to the sucrose. For example GB 2,105,338 describes such a process of preparation. A commercially available product is Neosugar ® (Beghin-Say, France).

Branched oligofructoses can also be used as functional ingredients in the present invention, they consist of a main chain and at least one side chain and comprise mainly fructose units. The main chain contains from 2 to 15 units and all the branching points are on fructose units. The side chains can be branched also, resulting in additional side chains. WO 91/13076, incorporated herein by reference, in the name of Raffinerie Tirlemontoise, Belgium, describes a production process of such branched oligosaccharides.

From another point of view, the present invention relates to functional food comprising the products used for their beneficial effects in the prevention of mammary carcinogenesis and the treatment of breast cancer as indicated herein above.

A functional food is any food product considered to be edible and comprising one or more food ingredients of which it is known that they provide the consumer with a physiological benefit including for example the prevention of disease, the treatment of disease, the activation of the biorhythm or the immune system.

Normally food has two purposes: feeding and sensorial pleasure. Functional food combines this with a supplementary physiological benefit. Functional food is not a pharmaceutical product and is different from a food product which naturally comprises a food ingredient known to have a physiological benefit. Functional food shall be designed such that when applied in a normal diet, the benefit on health and disease is obtained. Therefore the food ingredient with physiological benefit, defined herein as functional ingredient, is added in an amount which is significant from a preventive or therapeutic point of view.

Depending on the type of functional food, the functional ingredient is added depending on factors such as daily intake, food law regulations, organoleptic appreciations, sensorial pleasure, dose-related side effects. It will be evident for the man in the art to design and produce proper functional foods once he is aware of the functional ingredient properties of a food ingredient. The purpose of the functional food being such that this food product combines physiological benefit with good feeding and organoleptic pleasure.

Functional food, thus, conveniently allows to acquire the necessary and beneficial amounts of a functional ingredient without the need to take pills or syrups comprising the said ingredient.

Compared to food products naturally comprising one or another beneficial food ingredient, a functional food allows the consumer to obtain a higher concentration without the need to recur to an imbalanced daily diet which would occur when significant amounts are to be taken in. As only frequent intake can ensure that a functional ingredient exercises his effect of preventing or treating disease, the consumer can decide whether to take the same functional food regularly or to diversify his diet, depending on the consumer or patients feeding mode and wishes.

Functional food looks like everyday beverages, bakery, prepared meals, confectionary, dairy, dressings, spreads etc... but comprises a functional ingredient. The functional ingredient can either be added as a supplement or added to replace one or several of the normal ingredients.

Functional feed is administered to non-human mammals.

As a well known example of a familiar dietary component with functional ingredient properties one can cite calcium which can be added to everyday dairy products for example although they naturally comprise calcium. Calcium rich functional food can claim a beneficial effect on the prevention of osteoporosis.

Other familiar food ingredients with functional ingredient properties are certain dietary fibres reducing the risk of colon cancer for example, or oligosaccharides lowering serum triglyceride levels in hyperglycaemic conditions or improving the gut flora, or gut bacteria activating the immune system.

Inulin, oligofructose and their derivatives according to the invention are food ingredients which behave as dietary fibre in that first they are not digested in the small intestine as no human digestive enzyme exists that can break down the beta(2->1)-linkages, secondly because they enhance the passage through the gut. It is known that inulin as well as oligofructose are prebiotics. In other words that they are food ingredients known to modify the composition of endogenous gut microflora (N. Delzenne et al., Lebensm-Wiss u. Technol. 27,(1994),p 1) and especially stimulate the gut Bifidobacteria. They have reduced caloric value, can modify lipid metabolism (N. Delzenne et al., Am J. Clin. Nutr. 57(suppl), (1993),p 820S) and increase absorption of minerals such as Ca, Mg, Fe, Zn, and Cu.

The present invention exploits a thus far unknown beneficial physiological effect of inulin, oligofructose and their derivatives combined with the advantage of dealing with readily, safe and non toxic known food ingredients.

The functional ingredients according to the invention may be incorporated into food, feed or pharmaceutical products when it is in powder, liquid or cream form, according to processes readily known by the man in the art.

As an example WO 93/06744 in the name of Raffinerie Tirlemontoise, Belgium, enclosed herein by reference, describes a Rafticreaming ® process that can be used to prepare functional food products comprising inulin. The same process can be used to prepare creamy or cream comprising OTC or pharmaceutical compositions.

Both the preferred products, Raftiline ® and Raftilose ®, are commercially available in powder form and can therefore be added to for example powdered food or tablets.

The OTC or pharmaceutical compositions suited for oral administration are the ones known for such form of administration, for example, tablets, (coated or non coated), pills, capsules, solutions or syrups.

By way of exception it can be conceived to administer the products according to the invention rectally. Then, the prepared composition is presented in the form of suppositories.

The pharmaceutical compositions are prepared according to the methods generally applied by pharmacists and may include solid or liquid, non-toxic and pharmaceutically acceptable vehicles. The inclusion in galenic mediums can equally be foreseen.

The percentage of active product according to the invention can vary within very large ranges, only limited by the tolerance and the level of acquaintance of the patient to the product. The limits are particularly determined by the frequency of administration.

The criteria of tolerance establishing the limits are comparable with those for functional food or feed.

The present invention concerns also the use of the pharmaceutical composition according to the invention for the manufacture of a medicament destined for the prevention of carcinogenesis, preferably mammary carcinogenesis, and/or treatment of cancer, preferably breast cancer.

The present applicant has demonstrated that said composition may be used to reduce the kinetic of appearance of tumours, preferably breast tumours, to lower the intensity of malignant cancer, preferably breast cancer, and the yield of all tumours.

Unmistakable indications exist that it also lowers the degree of invasiveness and reduces metastasis of malignant cancer, preferably breast cancer. The incidence of cancer, especially breast cancer, and the duration of the latency period is influenced by these functional ingredients. The degree of malignancy of the appearing breast tumours appears to be lowered by the said functional ingredient.

The composition according to the invention is preferably used for negatively modulating carcinogenesis and for the prevention of cancer initiation, preferably breast cancer initiation.

The negative modulation of carcinogenesis is defined as any prevention of carcinogenesis that has at least one of the following effects:

- it allows to lengthen the latency period;
- it slows down the kinetics of appearance of malignant tumours;
- it lowers the incidence of cancer;
- it lowers the intensity of cancer;
- it lowers the degree of malignancy of appearing tumours;
- it lowers the degree of invasiveness of malignant cancer;
- it reduces metastasis of malignant cancer.

Incidence of cancer is defined as the number of individuals bearing tumours in one test group.

Intensity of cancer is the mean number of malignant tumours per individual bearing tumours.

Yield is the total number of, eventually malignant, tumours in one test group.

Tumours can be classified as benign or malign as far as the degree of malignancy is concerned.

Parameters for calculating the reduction of invasiveness and metastasis are expressed as metastasis incidence and incidence of tumours in other organs or tissues.

Modulation of carcinogenesis has, as defined herein, no direct relevance to multiphase/multistep carcinogenesis. Its effect can only be demonstrated by following the kinetics of cancers as they appear and/or by recording the incidence and yield of histologically characterised cancers. This terminology should broaden the view on carcinogenesis. The negative modulating properties of the functional ingredients according to the invention are particularly interesting in the context of the effects of more systematic procedures or experimental conditions such as chronic exposure to chemicals, dietary imbalances or surgery that disrupts metabolic or proliferative homeostasis. Even though such procedures are not essential for making an otherwise induced process fully carcinogenic, they can still influence its pathogenesis by creating conditions that speed up the kinetics of its development to malignancy and consequently increase or decrease the incidence and/or yield of cancer.

The present invention is illustrated by the following examples, without limiting it.

Rat mammary carcinogenesis as used in the examples is a well established model, closely mimicking the human disease, and allows to verify whether breast carcinogenesis can be manipulated by treatment of the host.

Especially the induction of breast cancer in Sprague Dawley rats by the target specific N-methylnitrosourea (MNU) carcinogen induces tumours with a latency period between 8 and 21 weeks and with an almost 100% final breast cancer incidence in untreated rats.

MNU can be given in a single 25 to 50 mg/kg body weight dose through subcutaneous injection. The susceptibility of the mammary gland to this specific carcinogen is age-related and should therefore be used for initiation between the age of 45 to 60 days of age. That is the age of sexual maturity. The Sprague Dawley rats used are known to be particularly susceptible for these testings. As it is known that high-fat conditions increase breast carcinogenesis, the diets fed to the rats must supply sufficient fat for normal development but without excess.

Calorie consumption is another factor influencing breast tumour incidence. Therefore the diet fed to the control group should be iso-caloric compared to the one fed to the test group and equal amounts should be consumed by both the control and the test animals.

Example 1: negative modulation of mammary carcinogenesis

1.1 tumour induction method

30 Sprague Dawley female rats, 45 days old and weighing +/- 100 g, are randomly divided into 4 groups. The animals of groups A and B (9 per group) receive a subcutaneous injection of 0,9% NaCl containing 50 mg/kg body weight N-methylnitrosourea (Serva, 30802)(MNU). The animals of groups C and D (6 per group) which are considered as controls are injected with the same volume of 0,9% NaCl solution. One week later, the powder diet AO3-UAR (INRA, France) of the animals of groups B and D is supplemented with 5% w/w Raftilose ® P95 (Raffinerie Tirlemontoise, Belgium) (OF). The concentration of OF in the diet is increased to 10% the second week and is finally 15% after the third week and until the end of the experiment. The animals of groups A and C receive successively the powder diet containing a supplement of 1%, 2,5% and 5% starch. This is an isocaloric diet as compared to the OF supplemented diet. Food and water are available at libitum.

Parameters monitored weekly are : body weight gain, diet and water consumption, faecal excretion. The size, number and position of putative mammary tumours are assessed by palpation.

At week 27, rats are anaesthetized with diethylether and killed by exsanguination. The organs (liver, kidneys, lungs, mammary glands) are macroscopically examined in situ and then weighed. The tumours are described (localisation, aspects) and measured. Tissue samples are taken and fixed in formalin for 3 weeks, then embedded in paraffine for further hematoxylin-eosin coloration and histological examination.

1.2. Results during the experiment

The diet consumption was not significantly different between the different groups. The dry weight of faecal excretion was not significantly different in OF treated rats as compared to rats receiving starch.

During the experiment according to example 1.1, the size , number and position of the tumours were evaluated using palpation and recorded as represented in fig 5 and 6; they give the tumour incidence (the number of rats bearing mammary tumours in one group) and the yield (total number of malignant tumours in one group).It can be seen from these data that the incidence and yield of mammary tumours is always lower in rats fed OF instead of starch.

1.3. Results at the end of the experiment

After the sacrifice at week 27, the tumours are macroscopically analysed and all the organs are checked for the putative presence of lesions. Histological examination allows to classify the tumours as benign or malignant. The incidence of metastases and tumours in other organs or tissues is noted. As shown in table 1, in the control group A, where 7 out of 9 rats are bearing tumours: 19 malignant mammary cancers , consisting mainly of low or mid-differentiated adenocarcinoma and 2 renal fibrosarcoma. One of those rats has an epidermal, cystic papilloma of salivary glands with no signs of malignity. The examination of the organs after sacrificing the animals revealed that the number of rats bearing cancer, the incidence, was the same for group B (MNU/OF), 7 out of 9 are bearing tumours. But the total number, or yield, of low or mid-differentiated adenocarcinoma is lower in group B than in group A: only 12 mammary adenocarcinoma are diagnosed, but neither renal tumours nor metastasis can be detected. The total volume of mammary tumours is almost 50% higher in the control group. The mean volume of mammary tumours is more or less the same in both groups, but lower in OF fed animals. The mean number of malignant mammary tumours per rat bearing cancer, the tumour intensity, at the time of sacrifice was lower in group B (MNU/OF) namely 1,7 compared to almost a double intensity in the control group A (MNU/starch), namely 3.

TABLE 1

Rats	Tumour					Metastasis	Volume (cm^3) of mammary tumours	
	Benign	Malignant					Total	Mean
		Mammary adenocarcinoma	other	yield	intensity			
Control-fed	1	19	2	21	3	2	132	6,9
OF fed	0	12	0	12	1,7	0	73	6,1

From the above it can be concluded that addition of oligofructose to the diet negatively modulates mammary carcinogenesis by slowing down the kinetics of appearance of breast tumours. Although the incidence looks the same, the mammary tumour intensity is lower.

Example 2: Negative modulation with oligofructose, inulin and pectin

2.1. tumour induction method

45 days old female Sprague Dawley rats weighing +/-100 g are purchased from Charles River, Germany. They are housed by 3 in suspended stainless steel cages under temperature and humidity control with a 12/12 h light/dark cycle. Food and water are available at libitum. After their arrival, the rats are adapted to new housing conditions during 1 week. At the age of 52 days the rats are initiated for mammary carcinogenesis by a single subcutaneous injection of 50 mg/kg body weight MNU (Serva, 3080 2) in 0,9% NaCl. 3 days after initiation, a period necessary to recover from MNU toxicity, the rats of the ad hoc experimental groups are given access to a semi-synthetic DIET (INRA, France) comprising 65% maize starch, 5% cellulose, 3% maize oil, 3% palmoil (not hydrogenated), 22% pure casein, 1,2% vitamins, 0,8% minerals and 0,13% methionine supplemented with 5% OF, inulin (Raftiline ®) or pectin for 5 days. The next five days a supplement of 10% of the same non digestible oligosaccharides or dietary fibre is given. Finally, until the end of the experiment, they will receive experimental diets supplemented with 15% OF, inulin or pectin.

Parameters examined during the test are: body weight starting from the adaptation period, palpation, measurement and description of the mammary tumours once every 2 weeks, starting 6 weeks after MNU injection, diet and water consumption and 24 h faecal excretion once every 6 weeks starting 6 weeks after MNU injection.

After sacrificing the animals the following parameters are determined: mammary tumour incidence, mammary tumour intensity and yield, mammary tumour diameters, determination of types of mammary tumours (benign or malignant), metastases incidence, incidence of tumours in other organs and tissues. Statistical analysis of the results are done according to relevant procedure.

Example 3: effect of oligofructose on initiation of breast carcinogenesis

The experimental protocol according to example 1.1 and 2.1 illustrates the protective effects of inulin and oligofructose on the promotion (phase II) and progression (phase III) of breast carcinogenesis.

In order to investigate the effect of oligofructose and inulin on all phases of breast carcinogenesis, including phase I of initiation, the following protocol is followed.

Sprague Dawley rats of 37 days of age are given a semi-synthetic diet (INRA, France) supplemented with 5% OF for 5 days, followed by 5 days of a 10% supplemented and subsequently 15% supplemented diet. Only then the rats are initiated with MNU at a dose of 50 mg/kg body weight in 0,9% NaCl and fed the 15% supplemented diet until the end of the experiment. The same parameters are being controlled as in example 2.1.

Example 4:

4.1. Origin of tumours, animals and products

TLT (Taper liver tumour) is a very aggressive hepatocellular, transplantable (in NMRI mices both intraperitoneally and intramuscularly) tumour of spontaneous origin in the liver of a Swiss Webster mouse and was used in tumour therapy and other experiments (Taper et al., 1966; Cancer Research 26, pages 143 to 148, Cappucino et al., Cancer Research 26, pages 689 to 694, 1966). All experiments are performed on young male mices.

The NMRI mices come from "Animalerie Facultaire", UCL, Brussels, Belgium.

Sodium ascorbate (vitamin C) and menadion sodium (bisulfite (vitamin K3) are supplied by Sigma, Inulin (Raftiline ®) and the oligofructose (FOS) is obtained from laboratories of Raffinerie Tirlemontoise, Tirlemont, Belgium.

4.2. Tumour transportation

10^6 TLT living cells are intraperitoneally implanted at day 0 into 10 young adult mice of ± 25 g body weight.

These animals are maintained on basal diet AO3 (UAR, Villemoisson sur Orge, France) and water at libitum.

4.3. Tumour treatment

Experimental groups (figure 1)		
N° of group	Treatment	N° of mice bearing i.p. transplanted tumour
1	Control (treated with starch and physiological NaCl solution)	10
2	CK3	10
3	FOS	10
4	FOS + CK3	10

Experimental groups (figure 2)		
N° of group	Treatment	N° of mice
1	Control	10
2	FOS	10
3	Inulin	10
4	FOS	10

Inulin, FOS and GK3 vitamins are orally administered once every second day from the 7th before I. M. tumour transplantation (at day 0) and then once a day after transplantation until the death of all control mice. Each experiment is performed three times.

Tumour growth is evaluated by measuring with calipers two dimensions (length and width) of tumours twice a week in sacrificed mices. Figures 7 and 8 show the tumour surface measured every four days.

The figures 7 and 8 show the reduction of intensity of TLT cancer with the use of the functional ingredients according to the invention.

The figure 8 also shows the synergic effect of oligofructose and vitamins.

Example 5

The experiments of example 4 are repeated with the use of EMT6 tumour cells injected intraperitoneally in normal mices instead of the TLT tumour cells.

Example 6

TLT tumour cells are intraperitoneally implanted into young adult mices as described in example 4.

Oligofructose and inulin have been injected intraperitoneally.

According to the SUDAN VIIb test, a slight increase of the number of TLT cells containing fat positive cytoplasmic vacuoles has been measured in the TLT treated cells 24 and 48 hours after injection of inulin and oligofructose.

Example 7

To determine synergic therapeutic effect, a pharmaceutical composition comprising Raftiline ® and a conventional chemotherapeutic product actively destroying malignant tumour cells is prepared.

A doxorubicine derivative is injected (20 mg / kg) to mices fed with oligofructose (Raftiline ®) as described in the example 4.

The mices (DBA_2) were previously inoculated with L1210 leukaemic tumour cells (10^4 cells at day 0).

Claims

1. Composition comprising a functional ingredient chosen among the group consisting of inulin, oligofructose and/or their derivatives, for use as a medicament.

2. Composition according to claim 1, characterised as being an OTC or a pharmaceutical composition comprising conventional vehicles.

3. Composition according to claim 1, characterised as being a functional food or feed possibly comprising additional food or feed ingredients.

4. Composition according to any of the preceding claims, characterised in that said inulin is a chicory inulin, preferably native chicory inulin.

5. Composition according to any of the preceding claims 1 to 3, characterised in that the said oligofructose is obtained by enzymatic hydrolysis of inulin from chicory, Jerusalem artichoke or dahlia, preferably of native inulin.

6. Composition according to any of the preceding claims, comprising one or more conventional chemotherapeutic products actively destroying malignant tumour cells.

7. Use of the composition according to any of the preceding claims, for the manufacture of a medicament destined for the prevention of carcinogenesis and/or treatment of cancer.

8. Use of the composition according to any of the preceding claims 1 to 6, for the manufacture of a medicament destined for the reduction of appearance kinetic of malignant tumour, for the reduction of cancer incidence, for the reduction of cancer intensity, for the reduction of tumours yield, for the degree reduction of cancer malignancy, for the degree reduction of invasiveness malignant cancer and/or for the reduction of cancer metastases.

9. Use of the composition according to any of the preceding claims 1 to 6, for the manufacture of a medicament destined for the prevention of cancer initiation, preferably for the prevention of breast cancer initiation.

10. Use of the composition according to any of the preceding claims 1 to 6, for the manufacture of a medicament destined for the prevention of mammary carcinogenesis and/or treatment of breast cancer.

11. Use of a composition comprising a functional ingredient chosen among the group consisting of inulin, oligofructose and/or their derivatives as a functional food.

12. Use according to claim 11 for the prevention of cancer initiation, the prevention of carcinogenesis and/or the treatment of cancer, preferably breast cancer.

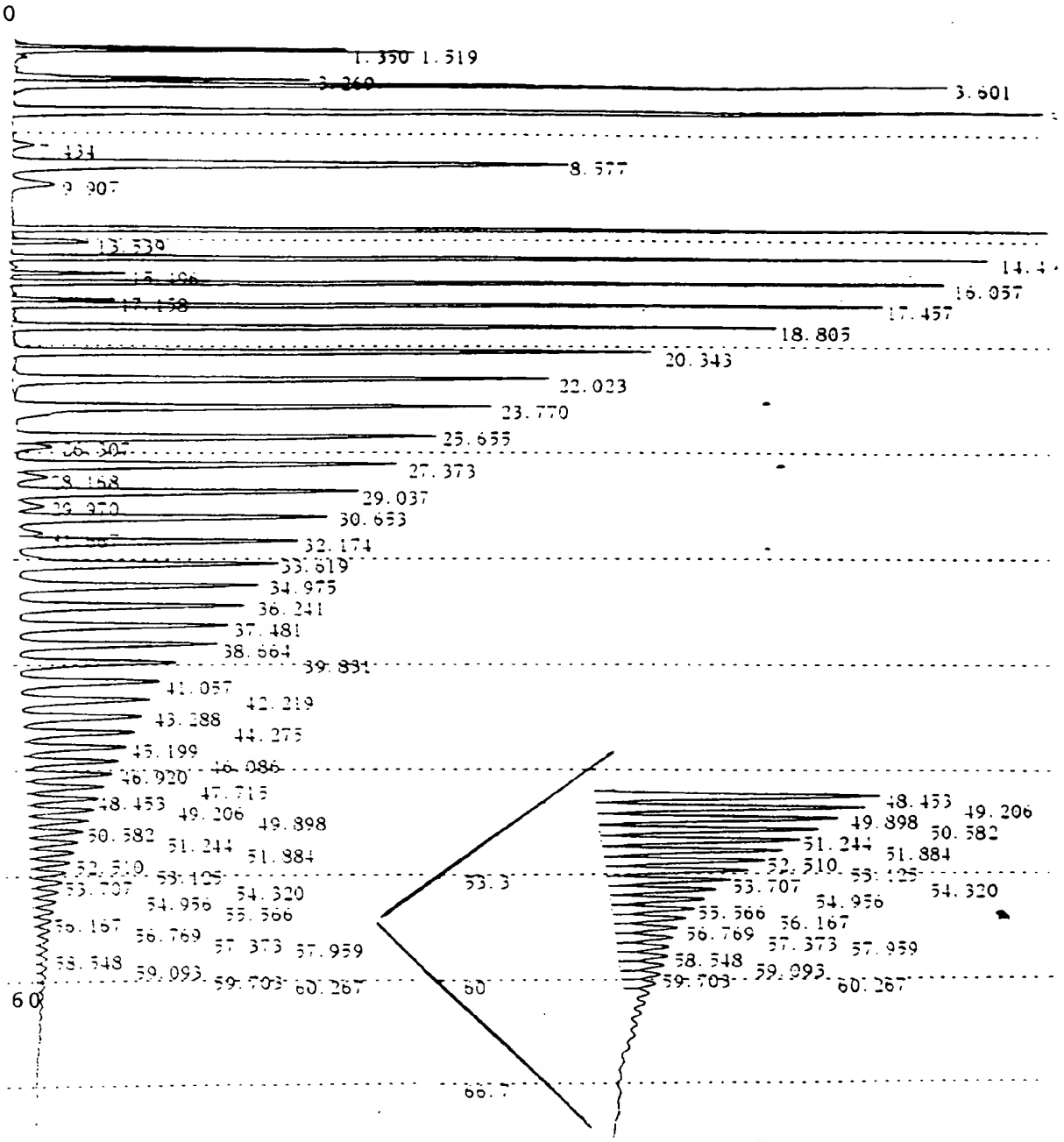
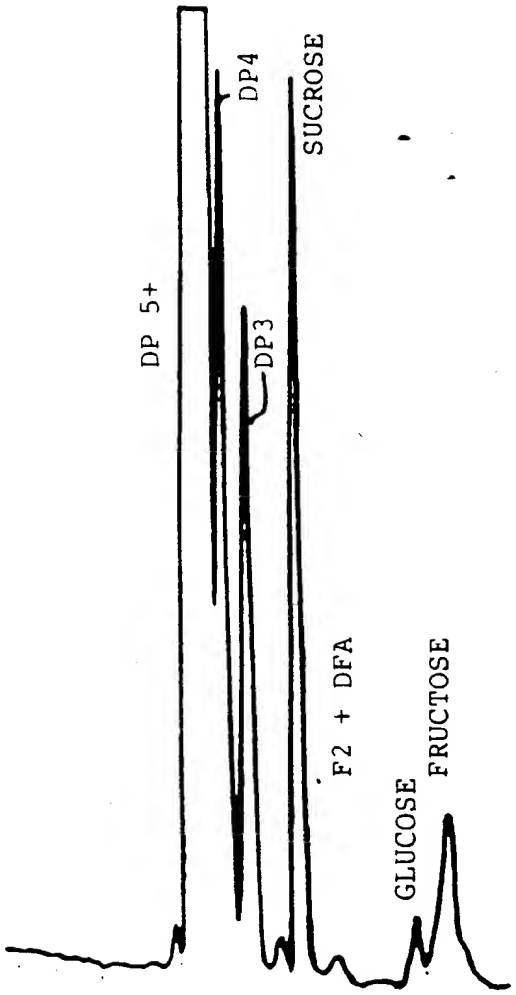


FIG.1

FIG.2



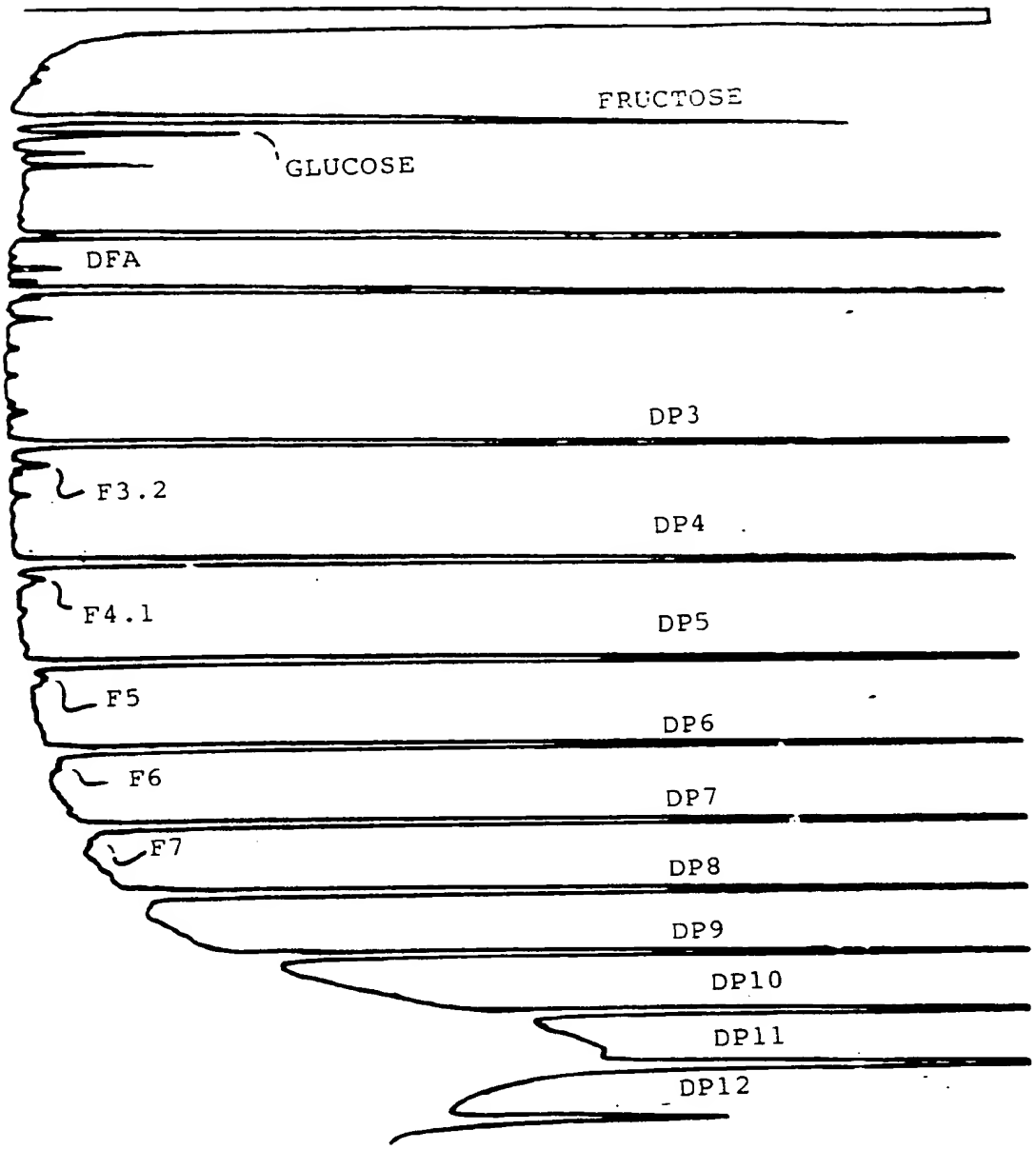


FIG.3

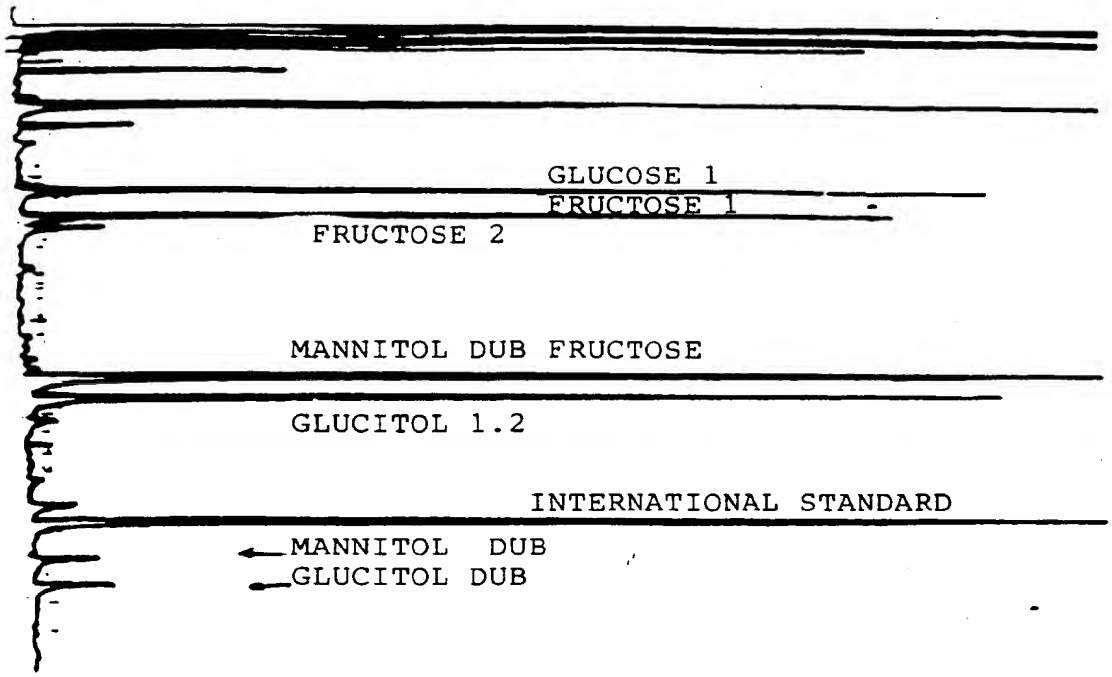


FIG.4

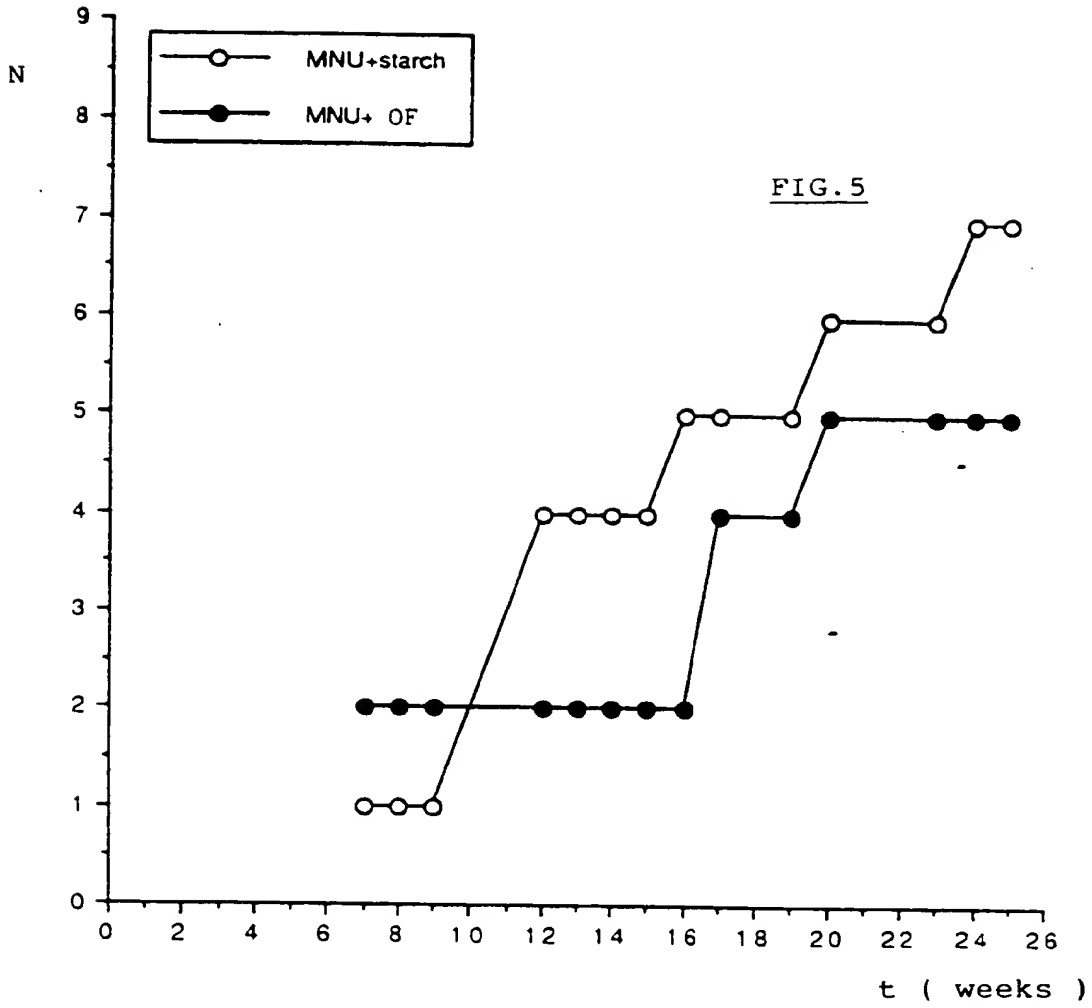


FIG.5

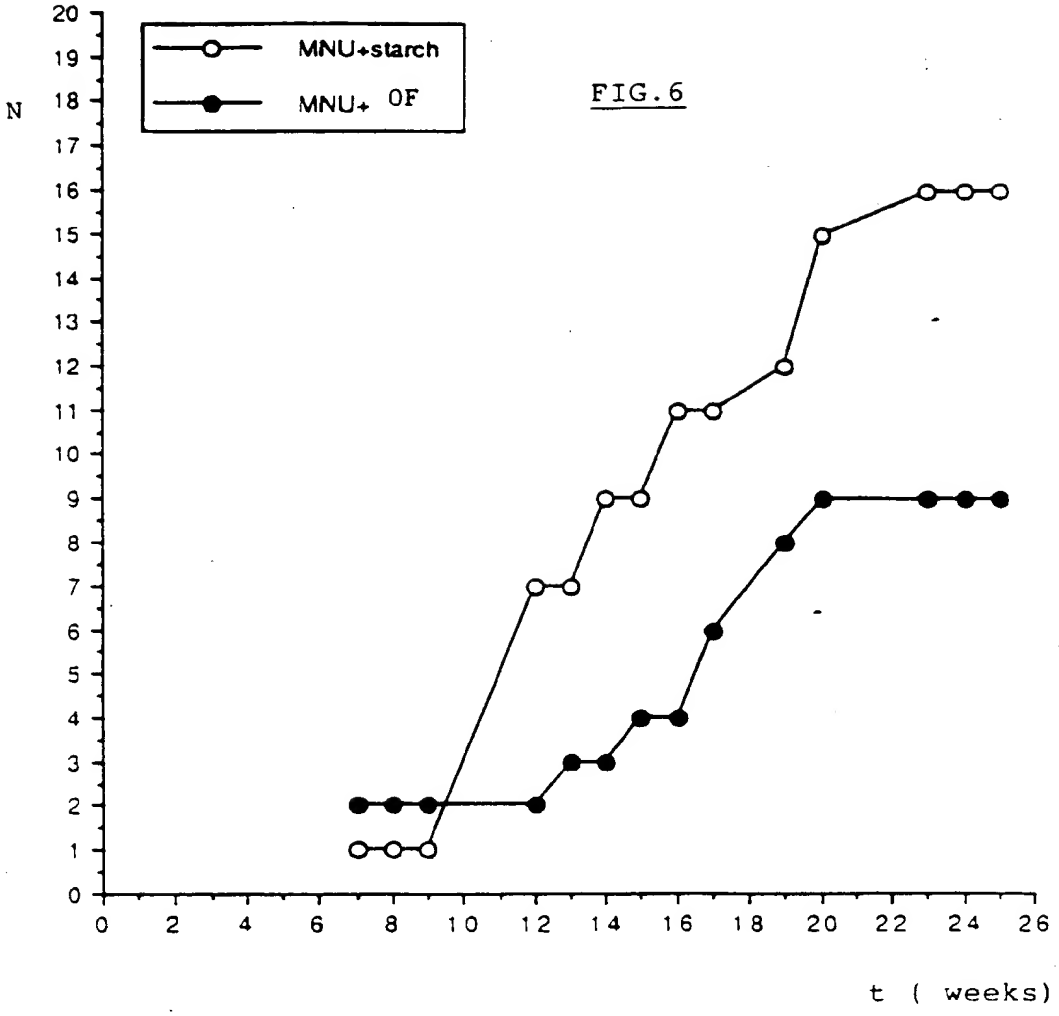


FIG.6

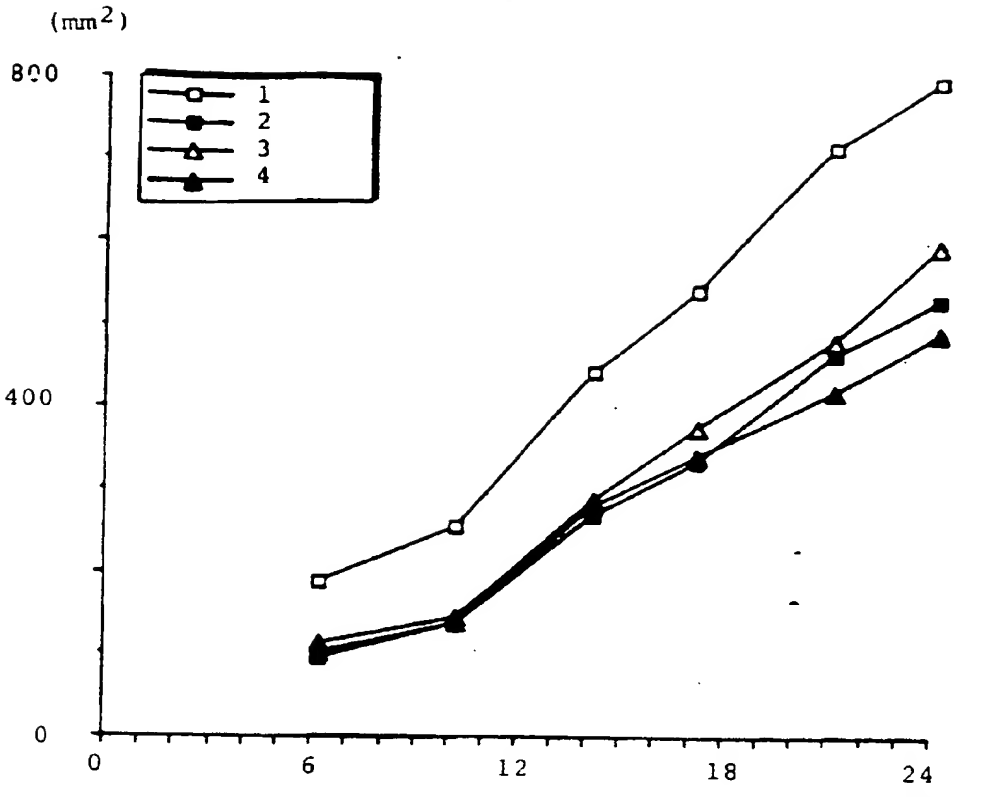


FIG .7

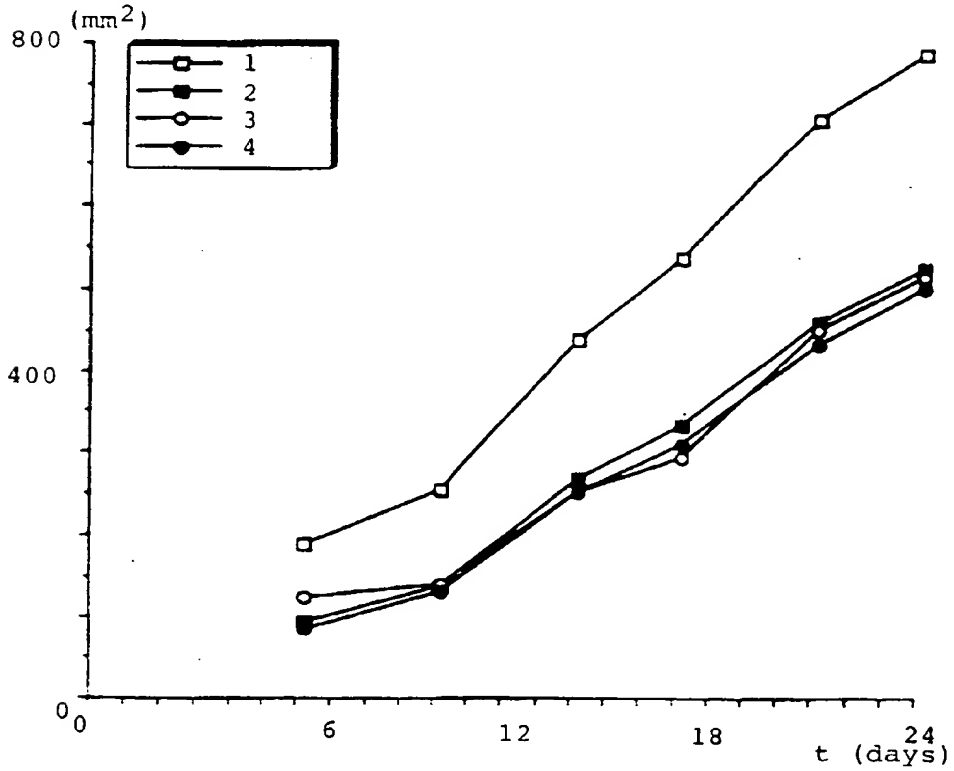


FIG.8

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EUROPEAN SEARCH REPORT

Application Number
EP 95 87 0069

DOCUMENTS CONSIDERED TO BE RELEVANT

	Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
1	X	DATABASE WPI Section Ch, Week 9033 Derwent Publications Ltd., London, GB; Class B04, AN 90-249702 & JP-A-02 172 921 (NITTO DENKO CORP) , 4 July 1990	1,2,7-9	A61K31/72
	Y	* abstract *	3-6, 10-12	

1	X	DATABASE WPI Section Ch, Week 8526 Derwent Publications Ltd., London, GB; Class B04, AN 85-156885 & JP-A-60 089 427 (DAINIPPON PHARM KK) , 20 May 1985	1,2,7-9	
	Y	* abstract *	3-6, 10-12	

1	X	DATABASE WPI Section Ch, Week 8431 Derwent Publications Ltd., London, GB; Class B03, AN 84-192836 & JP-A-59 110 621 (MEIJI SEIKA KAISHA) , 26 June 1984	1,2,7-9	TECHNICAL FIELDS SEARCHED (Int.Cl.6) A61K
	Y	* abstract *	3-6, 10-12	

1	X	DATABASE WPI Section Ch, Week 9105 Derwent Publications Ltd., London, GB; Class D13, AN 91-031795 & JP-A-02 295 462 (NAKAMURA T) , 6 December 1990 * abstract *	1,3,7-9, 11,12	

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	5 October 1995	Tzschoppe, D

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CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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EUROPEAN SEARCH REPORT

Application Number
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DOCUMENTS CONSIDERED TO BE RELEVANT

	Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
1	X	WO-A-87 02679 (THE AUSTRALIAN NATIONAL UNIVERSITY) 7 May 1987 * abstract * * page 1, line 1 - line 22 * * page 7, line 24 - page 9, line 30 * * page 21, paragraph 3 * * page 23, line 26 - page 24, line 19 *	1,2,6-9	
1	X	EP-A-0 280 474 (JOHNSON MATTEY, INC.) 31 August 1988 * claims 1-10; examples 6,7 *	1,2,6-9	
1	X	DE-A-35 39 951 (CESKOSLOVENSKA AKADEMIE VED PRAG) 15 May 1986 * claims 1,7,24 *	1,2,6-9	
2	D,Y	J.NATL. CANCER INST., vol. 83, no. 7, 1993 pages 496-501, L.A.COHEN ET AL. 'Modulation of N-nitrosomethylurea-induced mammary tumor promotion by dietary fiber and fat' * abstract *	10	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
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9	X	VET. HUM. TOXICOL., vol. 30, no. 2, 1988 pages 104-107, L. FISHBEIN ET AL. 'Fructooligosaccharides: A review' * page 106 *	1,3,5,11	

The present search report has been drawn up for all claims

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